REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
29-02-2012	Abstract	-

4. TITLE AND SUBTITLE
An in vitro model of blast-induced traumatic brain injury

5a. CONTRACT NUMBER
W911NF-10-1-0526

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS
Gwen B. Effgen, Matthew B. Panzer, Cameron R. 'Dale' Bass, David F. Meaney, Barclay Morrison III

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Pennsylvania
Office of Research Services
University of Pennsylvania
Philadelphia, PA 19104 -

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
58155-LS-MUR.6

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
Objectives: Blast-induced traumatic brain injury (bTBI) has risen to a new level of importance and is recognized to be a major cause of injuries to the brain. A simplified, free field blast-injury model would facilitate studies to correlate biological outcomes with blast-injury mechanics to generate novel tolerance criteria for bTBI.

Methods: Organotypic hippocampal slice cultures (OHSC) were cultured as previously described. OHSC were

15. SUBJECT TERMS
blast injury, shock tube, in vitro models, organotypic slice cultures, hippocampus, brain, neuron

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			David Meaney
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 215-573-2726

Report Title

An in vitro model of blast-induced traumatic brain injury

ABSTRACT

Objectives: Blast-induced traumatic brain injury (bTBI) has risen to a new level of importance and is recognized to be a major cause of injuries to the brain. A simplified, free field blast-injury model would facilitate studies to correlate biological outcomes with blast-injury mechanics to generate novel tolerance criteria for bTBI.

Methods: Organotypic hippocampal slice cultures (OHSC) were cultured as previously described. OHSC were plated onto porous membranes in supplemented Neurobasal medium. Culture medium was changed to conditioned full-serum medium starting 3-5 days following plating. OHSC were cultured under standard conditions (37 °C, 5% CO2) for 10-14 days. A 76 mm diameter shock-tube pressurized with helium was used to produce blast overpressures. A custom designed, water-filled receiver was maintained at 37°C. Cultures were placed into sealed bags with warmed culture medium and
placed in the receiver. Pressure transducers at the shock-tube exit and adjacent to the sample characterized loading of the sample. Control OHSC were secured in the receiver but were not exposed to blast. To test the neuroprotective potential of hypothermia, a group of cultures were injured with the temperature of the water in the receiver at 25 °C. All cultures were immediately returned to fresh culture medium and incubated. Propidium iodide (PI) fluorescence was used to measure tissue health prior to and at 1, 6, and 24 hours following injury. Cell death was determined for all OHSC regions as the percent area staining above an intensity threshold.

Results: This in vitro blast-injury model was capable of producing 175 kPa overspressures, which elicited diffuse cell death in OHSC that increased over 24 hours following blast. Control cultures experienced minimal cell death. Hypothermia was significantly neuroprotective and prevented cell death in cultures exposed to 175 kPa or 325 kPa overpressures.

Conclusions: Our in vitro blast-injury model recapitulates the translation of a shock wave in air, such as that produced by an explosive device, into a pressure wave similar to that within the skull-brain complex in vivo. Our results suggest that OHSC are vulnerable to and directly affected by blast-injury. OHSC exposed to blast at 25 °C were protected from the injury with minimal resultant cell death. To better prevent and treat bTBI, both the initiating biomechanics and the ensuing pathobiology must be understood in greater detail. Future studies will elucidate the tolerance of OHSC to various parameters of blast-injury as well as the mechanisms influential in this blast-induced cell death response. A well characterized, in vitro model of bTBI, in conjunction with animal models, will be a powerful tool in developing strategies to mitigate the risks of bTBI.

Title: An in vitro model of blast-induced traumatic brain injury.

Authors: Gwen B. Effgen, Matthew B. Panzer, Cameron R. 'Dale' Bass,
David F. Meaney, Barclay Morrison III

Objectives: Blast-induced traumatic brain injury (bTBI) has risen to a new level of importance and is recognized to be a major cause of injuries to the brain.[4,7] A simplified, freefield blast-injury model would facilitate studies to correlate biological outcomes with blast-injury mechanics to generate novel tolerance criteria for bTBI.

Methods: Organotypic hippocampal slice cultures (OHSC) were cultured as previously described.[1,2,5,6] OHSC were plated onto porous membranes in supplemented Neurobasal medium. Culture medium was changed to conditioned full-serum medium starting 3-5 days following plating. OHSC were cultured under standard conditions (37 °C, 5% CO_2) for 10-14 days. A 76 mm diameter shock-tube pressurized with helium was used to produce blast overpressures. A custom designed, water-filled receiver was maintained at 37°C. Cultures were placed into sealed bags with warmed culture medium and placed in the receiver. Pressure transducers at the shock-tube exit and adjacent to the sample characterized loading of the sample. Control OHSC were secured in the receiver but were not exposed to blast. To test the neuroprotective potential of hypothermia, a group of cultures were injured with the temperature of the water in the receiver at 25 °C. All cultures were immediately returned to fresh culture medium and incubated. Propidium iodide (PI) fluorescence was used to measure tissue health prior to and at 1, 6, and 24 hours following injury. Cell death was determined for all OHSC regions as the percent area staining above an intensity threshold.[1,2,3,5,6]

Results: This in vitro blast-injury model was capable of producing 175 kPa overspressures, which elicited diffuse cell death in OHSC that increased over 24 hours following blast. Control cultures experienced minimal cell death. Hypothermia was significantly neuroprotective and prevented cell death in cultures exposed to 175 kPa or 325 kPa overpressures.

Conclusions: Our in vitro blast-injury model recapitulates the translation of a shock wave in air, such as that produced by an explosive device, into a pressure wave similar to that within the skull-brain complex in vivo. Our results suggest that OHSC are vulnerable to and directly affected by blast-injury. OHSC exposed to blast at 25 °C were protected from the injury with minimal resultant cell death. To better prevent and treat bTBI, both the initiating biomechanics and the ensuing pathobiology must be understood in greater detail. Future studies will elucidate the tolerance of OHSC to various parameters of blast-injury as well as the mechanisms influential in this blast-induced cell death response. A well-characterized, *in vitro* model of bTBI, in conjunction with animal models, will be a powerful tool in developing strategies to mitigate the risks of bTBI.

1. Cater HL, Sundstrom LE, Morrison B 3rd. Temporal development of hippocampal cell death is dependent on tissue strain but not strain rate. J. Biomech. 2006: 39(15):2810-8.
2. Cater HL, Gitternman D, Davis SM, Benham CD, Morrison B 3rd, Sundstrom LE. Stretch-induced injury in organotypic hippocampal slice cultures reproduces in vivo post-traumatic neurodegeneration: role of glutamate receptors and voltage-dependent calcium channels. J. Neurochem. 2007: 101(2): 434-47.
3. Elkin BS, Morrison B 3rd. Region-specific tolerance criteria for the living brain. Stapp Car Crash J. 2007: 51: 127-38.
4. Long JB, Bentley TL, Wessner K, et al. Blast overpressure in rats: recreating a battlefield injury in the laboratory. J. Neurotrauma. 2009; 26(6): 827-40.
5. Morrison B 3rd, Cater HL, Wang CC, Thomas FC, Hung CT, Ateshian GA, Sundstrom LE. A tissue level tolerance criterion for living brain developed with a in vitro model of traumatic mechanical loading. Stapp Car Crash J. 2003: 47:93-105.
6. Morrison B 3rd, Cater HL, Benham CD, Sundstrom LE. An in vitro model of traumatic brain injury utilizing two-dimensional stretch of organotypic hippocampal slice cultures. J. Neurosci. Methods. 2006: 150(2):192-201.
7. Owens BD, Kragh JF, Wenke JC, et al. Combat wounds in operation Iraqi Freedom and operation Enduring Freedom. J. Trauma. 2008; 64(2): 295-99.